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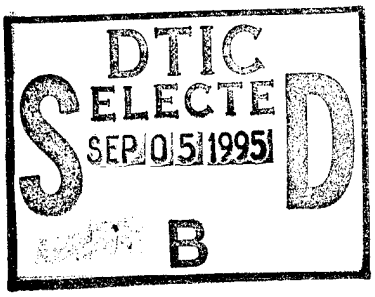

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184A1 human mammary epithelial cells (HMECs) form organotypic structures when plated on Matrigel. However, the organotypic structures differentiate into squamous, not glandular, epithelia. They can not be used to examine the functional significance of a polarized epidermal growth factor receptor system in a glandular epithelia. This experimental system may be useful for investigating cell-extracellular matrix interactions that control EGF-R expression. 184A1s and subclones of the 184A1 cell line express proteins necessary for structural and functional polarization on Transwells, but do not assemble these proteins into tight junctions. MCF-7 cells express ZO-1 and assemble the protein into tight junctions when plated onto Transwells. MCF-7s also segregate B-1 integrin into the basal-lateral membrane compartment. MCF-7 cells respond mitogenicly to EGF-R occupancy and may provide an experimental system with which to address the primary aim of my proposal: to determine if the loss of the correct spatial organization or inappropriate expression of the EGF-R system provides a growth advantage, enhances cell motility, or ochanges the differentiated state of normally organized epithelial cells.

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FOREWORD

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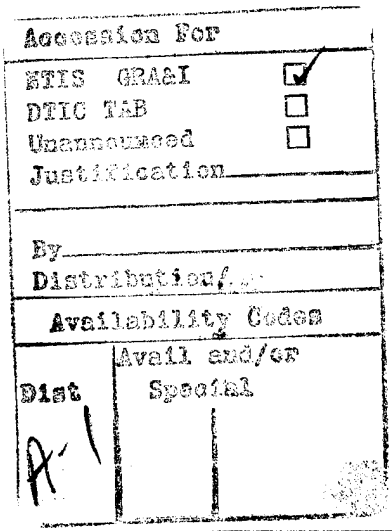

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INTRODUCTION:

Spatial Distribution of the EGF Receptor System in the Regulation of Breast Epithelial Cell Growth and Organization

The epidermal growth factor receptor (EGF-R) system is necessary for the motility, proliferation and differentiation of human mammary epithelial cells (HMECs) *in vitro*. Additionally, the EGF-R system displays a highly organized spatial distribution *in vivo*. Because the EGF-R system plays a central role in HMEC proliferation, it is reasonable to suspect that any defects in its regulation could lead to the clonal expansion of 'pre-malignant' cell populations. Such expanding clonal populations could give rise to cancerous clones. The spatial distribution of the EGF-R system is highly organized *in vivo*. *In vivo*, the receptor and one of its ligands, transforming growth factor alpha (TGF– α), are localized to the basolateral surface of mammary alveolar structures; on the other hand, epidermal growth factor (EGF) is synthesized and secreted from the apical side into the lumen of the alveoli. Regulation of the EGF-R system could be disrupted by removing the spatial restrictions which segregate one of the ligands from the receptor and/or the receptor from second messenger systems. I will investigate the spatial distribution of both the EGF-R and its ligands during the organization of HMECs on basement membranes (BM) *in vitro* and determine the consequences of disorganizing this distribution.

SPECIFIC AIMS:

1. Define the spatial distribution and expression levels of the EGF-R system in proliferating and spatially organized normal HMECs.

2. Determine whether a loss of the correct spatial organization or inappropriate expression of the EGF-R system provides a growth advantage, enhances cell motility, or changes the differentiated state of normally organized epithelial cells.

BODY OF REPORT

The HMECs I chose to use in this work are designated 184A1 and were isolated by Martha Stampfer as an immortalized derivative from the parental 184 cell line (1). They exhibit the ability to organize into organotypic structures when plated onto the extracellular matrix material Matrigel (extracted from Englebreth-Holm-Swarm murine sarcoma), previously shown in proposal. Histological sections of the organotypic structures stained with hematoxylin and eosin strongly suggest that after 3 weeks in culture on matrigel the 184A1s differentiate into squamous, rather than glandular epithelia as initial sections suggested. Identification of intracellular bridging, layering of cells and the secretion of cytokeratins are the ultrastructural basis upon which the organotypic structures have been identified as squamous in nature.

EGF-R levels have been examined during this squamous differentiation process and appear to decrease rapidly upon contact with the basement membrane and continue to decrease over a seven day period (figure 1). The mechanism of this regulation is unknown, but will be investigated further to determine if it is post-translational, post transcriptional or both. The involvement of specific integrin-extracellular matrix interactions in this process will also be investigated.

184A1s and subclones of 184A1s were plated onto tissue culture membranes as a way to induce epithelial polarization. Using membranes is a common experimental approach by which to investigate properties of polarized epithelia. 184A1s express the protein ZO-1, an integral component of the tight junctions that is characteristic of polarized epithelia; however when compared to the postive control, polarized MDCK cells, it is obvious that the protein is not part of a well formed intercellular juction (figure 2) (2,3,4). Different media and substratum conditions were used to induce tight junction formation, but no attempts have been successful thus far.

I have investigated the ability of a non-aggressive breast cancer cell line MCF-7 to functionally polarize as an alternative model to address my initial specific aims. The human colon cancer cell line, Caco-2, has served as an analogous system for those studying the structure and function of polarized intestinal epithelia (3,4). Initial immunofluorescence results strongly suggest that MCF-7 cells polarize when plated onto tissue culture membranes (Costar, Transwells) as determined by the distribution of ZO-1 and Beta-1 integrin. The distribution of both is consistent with a polarized epithelia (figure 3). MCF-7 cells are a classic example of an EGF-R autocrine cell; their proliferation is estrogen dependent which imparts much of its growth influence through induction of the EGF-R/TGF-α autocrine loop (5,6,7). MCF-7 cells may serve as an effective model for studying the functional importance of a polarized distribution of the EGF-R system.

CONCLUSIONS

1. Organotypic HMECs structures differentiate into squamous, not gladular, epithelia when plated on matrigel. This experimental approach may be useful for investigating cell-extracellular matrix interactions that control EGF-R expression. The mechanism will be detrmined to be post-translational and/or transcriptional, and further investigations will examine the role of laminin and ß-1 integrin in this process.

2. 184A1s and subclones express proteins necessary for structural and functional polarization on transwells, but do not assemble these proteins into tight junctions.

3. MCF-7 cells express ZO-1 and assemble the protein into tight junctions when plated onto transwells. MCF-7s also segregate ß-1 integrin into the basal-lateral membrane comparment. Further studies, including electron microscopy, immunofuorescence and measurments of electrical resistance will better define the degree of structural and functional polarization in MCF-7s.

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Appendix

Figure 1: Decreased EGF-R Expression During Organization on Matrigel as determined by western blot, equalamounts of protein were loaded in each lane; the control is cells grown on plastic

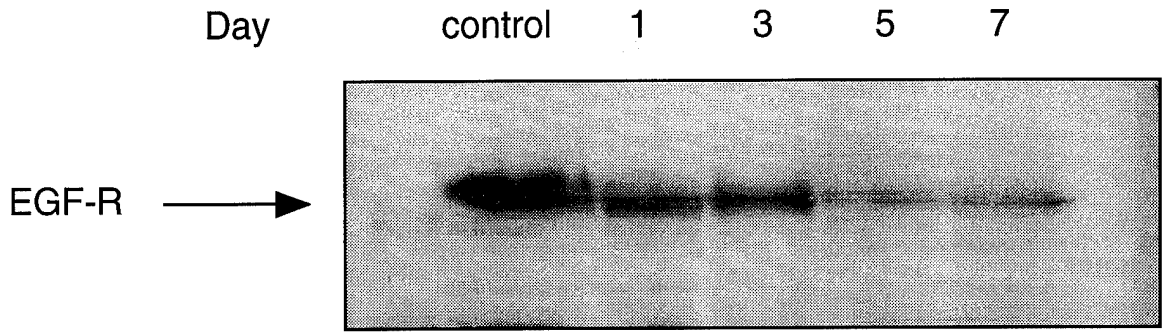
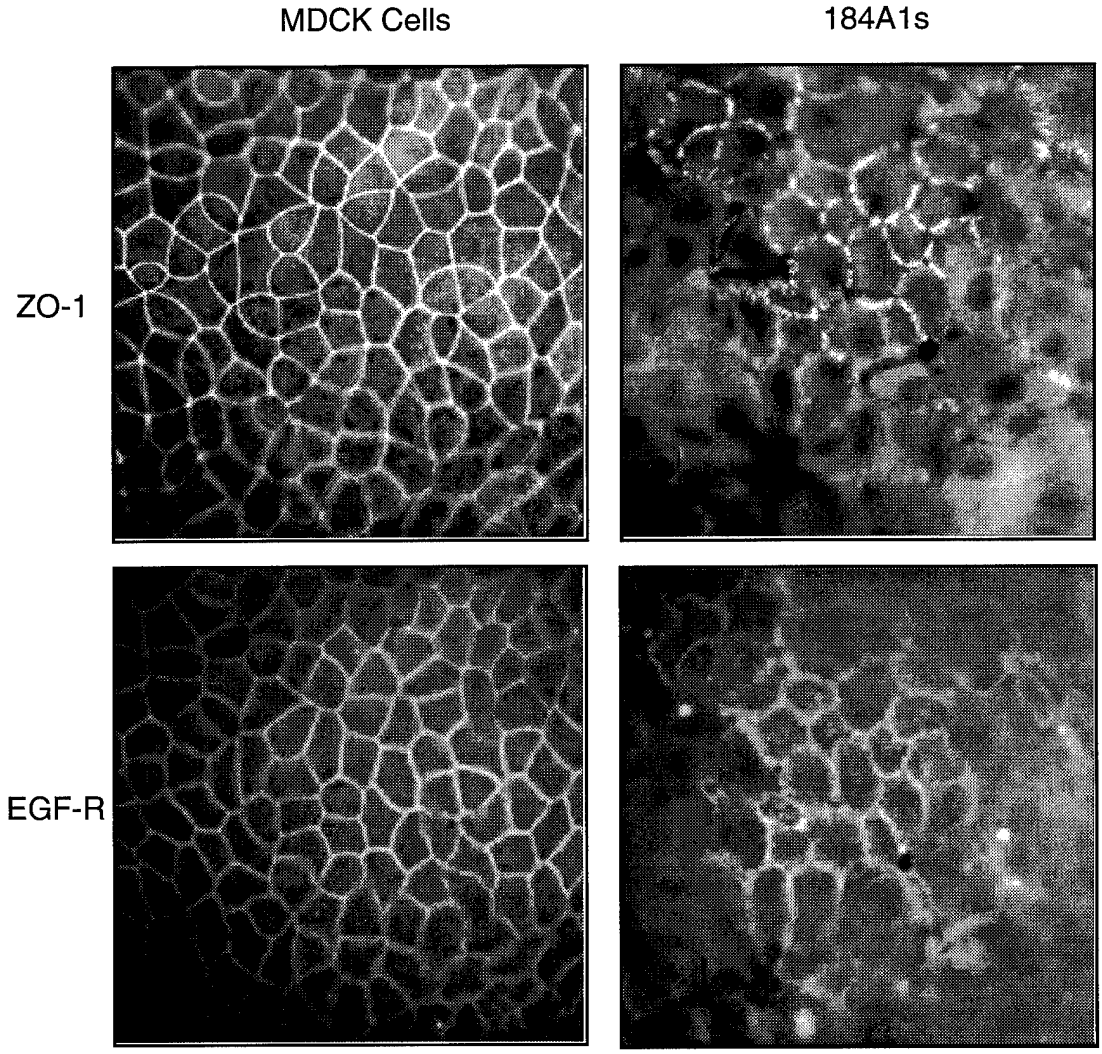


Figure 2: 184 HMECs Do Not Form Well Differentiated Polarized Epithelial Layers on Membranes



Appendix

Figure 3: Polarization of MCF-7, Compare to figure 2 and the localization of ZO-1 and EGF-R in MDCKs (positive controls)